

Review

Human tissue kallikreins as promiscuous modulators of homeostatic skin barrier functions

Azza Eissa^{1,2} and Eleftherios P. Diamandis^{1,2,3,*}

¹Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto M5G 1L5, ON, Canada

²Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto M5T 3L9, ON, Canada

³Department of Clinical Biochemistry, University Health Network and Toronto Medical Laboratories, Toronto M5G 1X5, ON, Canada

*Corresponding author

e-mail: ediamandis@mthsina.on.ca

Abstract

Human tissue kallikreins (KLKs) are the largest family of secreted serine protease endopeptidases encoded by 15 genes clustered on chromosome 19q13.4. Multiple KLK enzymes are co-localized in the upper stratum granulosum and stratum corneum of human epidermis, and in associated appendages such as hair follicle epithelia and sweat glands. Until recently, kallikrein proteolytic activity in the skin was exclusively attributed to KLK5 and KLK7. However, wider cutaneous roles of kallikreins became evident in recent years as the proposal of KLK proteolytic activation cascades emerged. We postulate that these proteolytic enzymes may serve as promiscuous mediators of different skin barrier functions, since they are capable of proteolyzing different substrates that govern skin desquamation, antimicrobial defense, and lipid permeability. Growing evidence now attests to potential kallikrein involvement in skin inflammation, pigmentation, and tumor suppression via their ability to target proteinase-activated receptor signaling pathways. Current knowledge on kallikrein roles in skin physiology and pathobiology is described in this review.

Keywords: activation cascade; corneodesmosomes; proteinase-activated receptors; serine protease inhibitors; skin diseases; stratum corneum.

Introduction

The human skin and its appendages execute an intricate web of functions that are vital for human survival. The skin protects the body from water loss, seasonal variation and UV radiation, and is the primary line of defense against xenobiotic infections. Approximately 60% of the worldwide population has some sort of a dermatological disorder, with 25% of this number requiring medical attention (Hadgraft, 2002). Clinical diagnosis of skin disorders is a difficult task, as aberrant skin manifestations

tend to underlie many unrelated local or systemic diseases. Skin diseases are difficult to treat as well, because of problems in dermal delivery through the persistent skin barrier wall created by the outermost skin layer, the stratum corneum (SC).

The SC is a 15- μ m-thick layer of 14–20 stacks of corneocytes, generated by a stratified epithelium that is renewed every 2–4 weeks. Mature human epidermis contains stratum basale (SB), stratum spinosum (SS), and stratum granulosum (SG) layers of epithelial cells known as keratinocytes, in addition to the outermost corneal layer. The formation of epidermal layers begins with commitment of a single layer of multipotent ectodermal progenitor cells to a keratinocyte cell fate in the lower SB. The undifferentiated keratinocytes forming the SB differentiate upon migrating upwards through the SS and SG, and are finally transformed into anucleated corneocytes in the SC layer.

Until recent years, the SC was viewed as a dead cell layer devoid of exciting metabolic activity, consistent with the early 1980s ‘brick and mortar’ model describing corneocytes as ‘bricks’ held together by an extracellular lipid ‘mortar’ (Elias, 1983). This view was discarded later since it describes a static structure for the dynamic SC and overlooks the fact that the main factors responsible for attaching corneocytes are corneodesmosomes, not lipids (Haftck, 2002; Kligman, 2006). With further research, it became evident that the SC tissue autoregulates several of its protective functions through persisting metabolic activities localized to its extracellular lipid compartment. SC interstices contain numerous secreted products of terminal keratinocyte differentiation, including structural proteins, lipids, proteases, lipid processing enzymes, and antimicrobial peptides, which are integral for homeostatic cutaneous processes such as skin desquamation, lipid permeability, and antimicrobial barrier formation. A list of some of the recurrent endogenous molecules that carry out persistent functions in SC interstices is shown in Table 1. Hence, the SC attains and maintains its versatile protective nature primarily through the actions of various structural proteins and enzymes secreted into its interstices, among which are human tissue kallikreins.

Tissue kallikreins are extracellular serine proteases secreted by granular keratinocytes into upper SG and SC interstices. The SC contains at least one aspartate protease, cathepsin D (Horikoshi et al., 1998, 1999), a cysteine protease named the stratum corneum thiol protease (SCTP) (Bernard et al., 2003), and several kallikrein serine proteases (Lundstrom and Egelrud, 1991; Brattsand et al., 2005; Komatsu et al., 2006b). To date, SC serine protease activity is attributed solely to human tissue kalli-

Table 1 Persistent proteins and hydrolytic enzymes in stratum corneum (SC) interstices.

SC interstitial molecules	Examples	Function
Structural proteins	• Corneodesmosomes: desmoglein 1, desmoglein 4, desmocollin 1, corneodesmosin	• Act as anchoring molecules that attach neighboring corneocytes
Proteases	• Serine proteases: kallikreins • Cysteine proteases: SC thiol protease • Aspartic proteases: cathepsin D	• Hydrolyze proteins into peptides (corneodesmosomes are common SC protease targets)
Sulfatases	• Steroid sulfatase	• Converts cholesterol sulfate into cholesterol
Lipases	• Acid lipase • Triglycerol lipase • Phospholipase A2	• Convert phospholipids and triglycerides into free fatty acids
Glycosidases	• β -Glucocerebrosidase	• Converts glycosylceramides into ceramides
Ceramidases	• Acid sphingomyelinase	• Converts sphingomyelin into ceramides

Table adapted from Haftek (2002).

kreins (Borgono et al., 2007), which have a broad spectrum of potential targets in the skin epidermal layers.

A wealth of literature demonstrated proteolytic functions for two serine proteases, KLK5 and KLK7, in the SC, previously dubbed SC trypsin enzyme (SCTE) and SC chymotrypsin enzyme (SCCE) (Lundstrom and Egelrud, 1988, 1991; Egelrud and Lundstrom, 1991; Brattsand and Egelrud, 1999; Ishida-Yamamoto et al., 2005; Descargues et al., 2006; Borgono et al., 2007). These kallikreins have been shown to activate antimicrobial peptides upon infection (Yamasaki et al., 2006) and to proteolyse intercorneocyte adhesion molecules resulting in corneocyte shedding (Caubet et al., 2004). It has recently been shown that more kallikreins are expressed in human SC and sweat, in addition to the formerly discovered KLK5 and KLK7 (Komatsu et al., 2005a,b, 2006b). Scientific investigations into the physiological functions of these additional kallikreins are ongoing, which are still largely unknown. Recent studies revealed that kallikreins may target proteinase-activated receptor (PAR) activation in keratinocytes, revealing the possibility of active kallikrein involvement in other facets of cutaneous biology.

The current status and future potential of kallikrein participation in the fields of cutaneous biology and human dermatology are discussed in this review. A better understanding of the regulatory mechanisms and physiological functions of tissue kallikreins in human skin may generate novel targets for skin disease diagnosis and therapeutics, as well as for the design of skin care products.

Kallikreins at a glance

Human tissue kallikreins (KLKs) are a family of 15 secreted serine proteases belonging to the chymotrypsin-like serine endopeptidase family S1, subclass PA(S) (Diamandis et al., 2000; Yousef and Diamandis, 2001). The name of the family is based on the 1930s identification of its first member, KLK1, in the pancreas, referred to as 'kallikreas' in Greek. This protease was shown to have kininogenase activity, whereby it cleaves kininogens to produce kinin peptides, which bind to kinin receptors, triggering several biological effects. Another kininogenase enzyme expressed solely in the liver and encoded by a single gene on chromosome 4q35 was subsequent-

ly discovered and named a kallikrein as well, based on its activity. However, based on the fact that these two kallikrein proteases share no genomic or proteomic structural homologies, investigators designated these enzymes to two separate categories, whereby the kallikrein encoded by chromosome 19q13.4 was dubbed human tissue kallikrein (KLK1) and the kallikrein encoded by chromosome 4q35 was dubbed human plasma kallikrein (KLK1B) (Lundwall et al., 2006).

During the late 1980s, two additional tissue kallikrein genes (*KLK*) were discovered in the same genomic vicinity as *KLK1*; the human glandular kallikrein (*KLK2*) and the prostate-specific antigen (*PSA, KLK3*) (Borgono et al., 2004). These two kallikreins exhibited very little to no kininogenase activity, although they shared genomic and proteomic homologies with *KLK1*. Accordingly, the traditional definition of a tissue kallikrein being a kininogenase enzyme acting on high-molecular-weight substrates to produce bioactive kinins was modified and the term tissue kallikrein was introduced to define serine protease enzymes encoded by genes on chromosome 19q13.4 and characterized by extensive structural homologies to *KLK1* at both the DNA and protein level, regardless of their proteolytic activities. *KLK1*, 2, and 3 and their gene products were referred to as classical tissue kallikreins, as they were shown to share a loop region found in rodent kallikreins and believed to be important for enzyme substrate specificity. The remaining 11 non-classical *KLKs* do not have this loop and hence are postulated to have diverged further from rodent kallikrein genes during evolution. In addition to mouse and rat, kallikrein gene families have been identified in the chimpanzee, dog, pig, and opossum mammalian species (Elliott et al., 2006).

The last decade of the 20th century witnessed active growth in kallikrein research as the *KLK* gene locus was fully characterized, expanding the human tissue kallikrein family from three to 15 genes and a pseudogene (*Ψ KLK1*), tandemly mapped to a contiguous cluster of approximately 400 kbp on chromosome 19q13.4, forming the largest contiguous protease gene cluster in the human genome (Yousef et al., 2000; Clements et al., 2001; Elliott et al., 2006). The most recent nomenclature of the kallikrein family refers to *KLK1* as human tissue kallikrein, and the remaining *KLKs* are dubbed kallikrein-related peptidases (Lundwall et al., 2006).

Kallikreins share a high degree of genomic and proteomic homology. All *KLK* genes contain five coding exons of similar sizes and a conserved intron-phase pattern of I-II-I-0 (Yousef and Diamandis, 2001). Approximately 82 *KLK* mRNA forms have been reported, as each *KLK* gene has at least one alternative splice variant (Kurlender et al., 2005). *KLK* genes contain both 5' and 3' untranslated regions of varying length, except for the classical *KLKs*. Alternatively, *KLK* proteins have a characteristic multi-domain structure consisting of an amino-terminal pre-peptide, a pro-peptide essential for maintaining the pro-*KLK* protein in a latent form, and a catalytic domain containing a highly conserved triad of histidine (H), aspartic acid (D), and serine (S) amino acids (Yousef and Diamandis, 2001).

KLKs are secreted as pro-*KLK* zymogens upon removal of their pre-peptide signal. Cleavage of the pro-peptide induces a conformational change in the active site and substrate pocket, resulting in extracellular activation of the mature enzyme (Borgono and Diamandis, 2004). Pro-*KLK* activation is a key regulatory process postulated to occur via a proteolytic activation cascade similar to those for the coagulation, fibrinolysis, and complement system cascades (Yoon et al., 2007). Once active, kallikreins act via a serine-directed nucleophilic attack mechanism to hydrolyze peptide bonds of target substrates, resulting in substrate activation, inactivation, or degradation. The majority of *KLK* proteins have an acidic Asp residue at position 189, or Glu189 in the case of *KLK15*, in their substrate-binding pocket, allowing them to interact with basic arginine or lysine residues in their target substrates and conferring trypsin-like substrate specificity. On the other hand, *KLK3*, 7, and 9 function as chymotrypsin-like serine proteases, as they contain Ser189, Asn189, and Gly189 in their substrate-binding pocket, respectively, accommodating bulky non-polar amino acids such as tyrosine and phenylalanine. Substrate specificity has been experimentally verified for all kallikreins apart from *KLK9*, 10, and 12 (Borgono et al., 2004).

Kallikrein expression and activity are tightly controlled by transcriptional and post-translational mechanisms owing to their irreversible proteolytic activity towards substrates. Kallikrein expression is modulated by steroid hormone and DNA methylation epigenetic regulation, whereas proteolytic activity is regulated by zymogen activation, endogenous inhibitors, allosteric regulation, and auto-degradation (Borgono and Diamandis, 2004). Some kallikreins have been shown to target kallikrein-kinin, urokinase plasminogen activator, matrix metalloprotease (MMP), and PAR pathways (Borgono and Diamandis, 2004; Borgono et al., 2004), although many of the signaling mechanisms remain to be elucidated for the non-classical *KLKs*.

A wide range of methodologies have been used to study *KLKs*, including RT-PCR for direct detection of *KLK* transcripts in human tissues and ELISAs to measure secreted protein levels in biological samples (Paliouras and Diamandis, 2006). Tools such as synthetic fluorogenic substrates, phage display, combinatorial peptide-based libraries, mass spectrometry, and bioinformatics are used to identify novel kallikrein substrates and inhibitors, and to determine *in vitro* and *in vivo* cleavage sites.

Gene knockout mice are also being used to study kallikrein functions *in vivo*.

Kallikreins are expressed in a wide range of tissues at varying mRNA and protein levels. *KLK* protein distribution was recently quantified by *KLK*-specific ELISAs, which allowed *KLK* categorization into three classes based on their tissue abundance pattern. The first class contains highly localized *KLKs*, such as: *KLK2* and 3 in the prostate; the second class includes *KLK5*, 6, 7, 8, and 13 localized mainly to three to five tissues; the remaining *KLKs* (1, 4, 9, 10, 11, 12, 14, and 15) belong to a third category of promiscuous kallikreins expressed in a wide range of tissues (Shaw and Diamandis, 2007).

The growing interest in the *KLK* enzyme family springs partially from their strong standing as potential serological cancer biomarkers, as they are differentially expressed in various malignancies. *KLK3* or PSA is a reliable prostate cancer biomarker currently in clinical use, for which levels >4–10 $\mu\text{g/l}$ in serum correlate positively with cancer occurrence. To date, at least 13 kallikreins are being investigated as potential biomarkers for different cancer types (Emami and Diamandis, 2007). Despite their clinical significance, kallikrein functions remain largely elusive. Emerging evidence links kallikreins to various non-cancer-related physiological processes, such as semen liquefaction, extracellular matrix (ECM) degradation, brain neurodegeneration, and immunophysical skin barrier formation. Physiological roles have been demonstrated for *KLK5* and 7 in skin desquamation and antimicrobial peptide processing, for *KLK2*, 3, 5, and 11 in semen liquefaction, for *KLK6* in ECM degradation, and for *KLK8* in brain neurodegeneration.

Kallikrein expression in the skin

Multiple kallikreins are expressed, in active *KLK* and inactive pro-*KLK* forms, in the skin epidermis and its associated appendages (Komatsu et al., 2005b). Kallikreins have been detected by immunohistochemistry in glandular epithelia secretions, confirming their extracellular localization (Petraki et al., 2006). In the epidermis, multiple *KLKs* co-localize in the upper SG and SC. *KLKs* such as *KLK5*, 7, and 14 have been extracted in active forms from SC tissues (Ekholm et al., 2000; Brattsand et al., 2005). Kallikrein transcripts and/or proteins have been detected by RT-PCR and immunostained in the SC, upper SG, sebaceous glands, eccrine sweat glands, hair follicles, and nerves (Komatsu et al., 2003, 2005b). *KLK* mRNA expression in the inner and/or outer root sheath of hair follicle epithelia (Komatsu et al., 2003), suggests *KLK* involvement in hair development. *KLK* mRNAs and proteins are also intensely expressed in the basal layer of undifferentiated sebocytes (Komatsu et al., 2003), indicating their potential participation in sebaceous gland differentiation and sebum formation (Komatsu et al., 2005b). Moreover, *KLKs* such as *KLK6*, 8, and 13 are found in the inner lumen of sweat gland ducts and hence are expected to be secreted in sweat (Komatsu et al., 2005b). Indeed, the expression of these three kallikreins in human sweat and surface corneocytes was confirmed

recently by ELISA quantification, verifying previous immunohistochemistry results (Komatsu et al., 2006b).

Expression of KLK3 and 9 proteins has not been observed in the epidermis (Komatsu et al., 2005a), indicating that epidermal chymotrypsin-like serine protease activity is solely due to KLK7. In addition to the chymotrypsin-like KLK7, seven trypsin-like kallikreins (KLK5, 6, 8, 10, 11, 13, and 14) have been detected in human SC and sweat from different body regions (Komatsu et al., 2006b). As shown in Table 2, Komatsu and colleagues detected a broad range of SC trypsin-like KLK levels, with the most abundant KLKs (KLK8 and 11) at levels approximately 200-fold higher than the least abundant ones (KLK14 and 13). Levels of KLK8 and 11 are similar in the SC, but KLK8 concentrations are significantly higher in sweat, representing up to 60% of the total trypsin-like KLK levels, Table 2. In a different study, Komatsu et al. (2005a) demonstrated that the total trypsin-like KLK concentration in the SC is approximately double the total chymotrypsin-like concentration, although this does not signify a higher trypsin-like activity. Interestingly, the total SC chymotrypsin-like level was consistent among different age groups, whereas the total trypsin-like concentration declined with age increase from 30 to 70 years old, revealing trypsin-like KLK involvement in skin aging (Komatsu et al., 2005a).

Profiling of epidermal kallikreins based on enzyme activity is difficult, as activity results vary depending on the assay type used (Brattsand et al., 2005). Casein zymography analysis of SC tissue extracts indicates that KLK5 and KLK7 are the major active KLKs in the SC, whereas chromogenic peptide substrate studies attribute up to 50% of trypsin-like activity to KLK5, and the majority of the remaining activity to KLK14 (Brattsand et al., 2005). Recently, another study indicated that KLK14 accounts for 50% of the overall SC trypsin-like KLK activity (Stefansson et al., 2006), despite being identified as a minor SC trypsin-like kallikrein (Table 2). Hence, less abundant KLKs in the SC may be catalytically more active than the abundant ones, although this requires further resolution. Furthermore, quantification of epidermal KLKs based on enzyme activity is difficult because KLK

activity in the epidermis is regulated by different endogenous and environmental factors.

Epidermal regulation of tissue kallikreins

Proteolytic activation cascade

Pro-kallikreins are believed to be activated in a step-wise manner involving an activation cascade, in which the active form of one kallikrein catalyzes activation of the next pro-KLK. The occurrence of such a cascade in the skin is supported by the co-expression of multiple KLKs in upper epidermal layers and sweat at varying concentrations (Table 2), and by the ability of some of these KLKs to auto-activate and activate other pro-KLKs (Brattsand et al., 2005; Yoon et al., 2007). A kallikrein may take on the role of initiator, propagator, and/or executor within the cascade, depending on its concentration, specificity, and activity level. However, minute amounts of the initiator are sufficient to trigger the whole process owing to its catalytic nature.

Initially, kallikrein actions in the skin were exclusively attributed to KLK5 and KLK7; however, wider kallikrein roles in the skin were identified as the concept of a catalytic activation cascade emerged. *In vitro* kinetic studies demonstrated that pro-KLK5 is activated by KLK14 and KLK5 itself, and active KLK5 activates pro-KLK7 and pro-KLK14, Figure 1 (Brattsand et al., 2005). Recently, Yoon et al. (2007) characterized the first extensive KLK activome upon examining the hydrolysis of 15 pro-KLKs by 12 mature KLKs, excluding KLK9, 10, and 15. Their novel findings allow expansion of the epidermal KLK activation cascade to include two additional KLK members known to be expressed in the SC, KLK6 and 11, in which pro-KLK11 is activated by itself and KLK6, and pro-KLK14 is activated by KLK11 (Figure 1).

Since at least eight kallikreins are present in human SC and sweat (Table 2), more cross-talk among KLKs and/or other epidermal proteins may occur, as yet undisclosed. The contribution of KLK8, 10, and 13 to the skin activation cascade remains to be elucidated. Furthermore, the characterization of initiators, propagators, and executors in the KLK proteolytic activation cascade poses a challenge that remains to be solved, although KLK5 is believed to be the cascade initiator (Brattsand et al., 2005). Current knowledge on the epidermal KLK proteolytic activation cascade in the skin is illustrated in Figure 1.

Lamellar granule trafficking

Lamellar granules (LGs), or lamellar bodies, are epidermal secretory granules that deliver cargos synthesized in upper granulocytes to SC interstices, including kallikrein serine proteases. In addition to epidermal transport and release of contents into intercellular spaces, LGs function as cargo storage sites (Braff et al., 2005). Accumulating literature suggests that LGs are in fact branched tubular structures based on visualization by cryotransmission electron microscopy, which clarifies earlier suggestions that LGs are simple, discrete, round, disk-like, or oblong structures visualized by classical electron microscopy

Table 2 Relative mean percentages of trypsin-like kallikrein levels measured by ELISA in stratum corneum (SC) and sweat samples from different body regions (modified from Komatsu et al., 2006b).

Protein	Relative mean percentage (%)	
	SC	Sweat
KLK8	40	61
KLK11	40	23
KLK5	10.6	10.5
KLK10	6.2	3.0
KLK14	1.4	1.1
KLK6	1.3	1.5
KLK13	0.5	0.9

The percentages represent the average of the fraction of each trypsin-like KLK to the total trypsin-like KLK amount measured by ELISA quantification in forearm, abdomen, back, and thigh SC tissues and in samples of facial, axillary, and abdominal sweat.

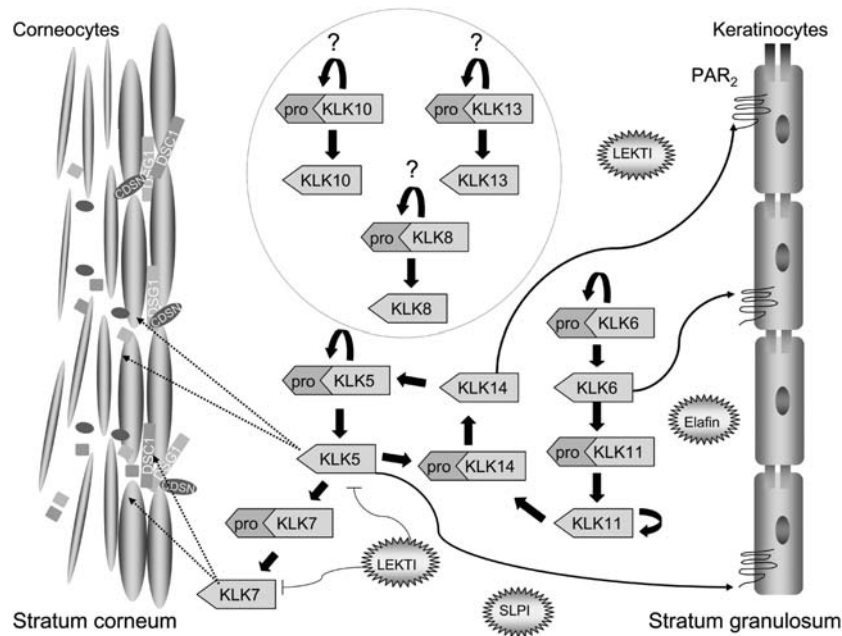


Figure 1 Kallikrein proteolytic activation cascade leading to corneodesmosome degradation and corneocyte shedding. Known activation interactions between the eight kallikreins (KLK5, 6, 7, 8, 10, 11, 13, and 14) expressed in the stratum corneum forming a tissue-specific activation cascade are indicated with solid black arrows; unknown interactions are denoted with question marks at the top. Upon activation, KLKs (KLK5 and KLK7) can target corneodesmosomes (DSG1, DSG4, DSC1, and CDSN) leading to skin desquamation or (KLK5, 6, and 14) can target PAR₂ activation in keratinocytes. Epidermal serine protease inhibitors (LEKTI, elafin, and SLPI) regulate KLK activity in the epidermis.

(Norlen et al., 2003; Ishida-Yamamoto et al., 2004). According to the membrane folding model, LGs are tubular continuations of the same membrane structures as granular keratinocyte *trans*-Golgi networks (TGN) to the interstices of the SG/SC border (Norlen et al., 2003). LGs fuse with the apical membranes of uppermost granulo-cytes, releasing their contents into extracellular spaces (Ishida-Yamamoto et al., 2004). Secretion from the uppermost differentiated granular keratinocytes allows KLKs to be delivered in close proximity to target substrates, such as corneodesmosomes, regulating their degradation, which results in skin desquamation. Cargo molecules, such as KLK5, 7, and 8, the epidermal protein lympho-epithelial Kazal-type inhibitor (LEKTI), and target substrate corneodesmosin (CDSN), are separately co-localized and transported in different LG vesicles before their release into the SG/SC interface (Ishida-Yamamoto et al., 2004, 2005). Separate LG transport averts any possible premature enzyme activity among cargos, such as proteolysis of CDSN by KLK5 or KLK7, which is known to occur at pH 5.5, similar to the environmental pH of LGs (Ishida-Yamamoto et al., 2004). In addition to transport, LGs regulate the time and location of cargo secretion. For example, it has been shown that LEKTI inhibitor is secreted earlier than KLKs into the superficial SG layer, whereas KLK5 and 7 are secreted into SC interstices (Ishida-Yamamoto et al., 2005). The exact mechanism for LG sorting, transport, and cargo secretion is not fully understood, but selective cargo aggregation and condensation have been suggested (Ishida-Yamamoto et al., 2004). Nonetheless, the temporal and spatial transportation and secretion of KLKs by LGs are critical regulatory events controlling epidermal kallikrein expression. Negative feedback loops regulating LG secretion of KLKs

may also occur in the epidermis, as hyperactive kallikreins have been reported to decrease LG secretions (Hachem et al., 2006).

Epidermal substrates and inhibitors

Numerous skin-specific kallikrein substrates and inhibitors are co-expressed with kallikreins in human SG and SC layers. Co-localization with myriad substrates in the SC, including hCAP18 and LL37 cathelicidin antimicrobial peptides, and desmoglein (DSG) 1, DSG4, desmocollin (DSC) 1 and CDSN corneodesmosomal cadherins, allows for their efficient proteolysis by kallikreins. On the other hand, co-localization with epidermal serine protease inhibitors, such as LEKTI, elafin, and secretory leukocyte protease inhibitor (SLPI), results in regulation of KLK activity. Akin to other serine proteases, kallikreins can be inhibited or trapped by forming a stable covalent complex with endogenous members of the superfamily of serine-protease inhibitors (serpins) such as α_1 -antitrypsin (Borgono and Diamandis, 2004), whereby the serpin acylates the protease active serine, resulting in a conformational change of the serpin reactive center and destruction of the protease active site (Huntington et al., 2000; Shin and Yu, 2002). Elafin and SLPI do not inhibit KLK5, 6, 13, and 14 (Borgono et al., 2007), although these inhibitors have been implicated in KLK7 inhibition and corneocyte shedding *in vitro* (Franzke et al., 1996). Alternatively, several KLKs, including KLK5, 6, 7, 13, and 14, are inhibited by LEKTI inhibitory domains (Egelrud et al., 2005; Schechter et al., 2005; Borgono et al., 2007; Deraison et al., 2007). Similar to KLKs, LEKTI is expressed in normal SC, SG and skin appendages (Bitoun et al., 2003; Raghunath et al., 2004). LEKTI is a reversible inhibitor of

1064 amino acids encoded by the *SPINK5* (serine protease inhibitor Kazal type 5) gene and organized into 15 serine protease inhibitory domains (D1–D15) (Bitoun et al., 2003). The full-length protein is an inactive inhibitor of KLKs. Its intracellular cleavage by furin generates single or multidomain inhibitory fragments that are secreted by keratinocytes to inhibit KLKs (Deraison et al., 2007).

LEKTI domains display distinct inhibitory profiles, as they are selective towards KLKs (Egelrud et al., 2005; Schechter et al., 2005). A recent study of the interaction of three kallikreins (KLK5, 7, and 14) with recombinant LEKTI fragments that represent epidermal LEKTI forms (D1, 5, 6, 8–11, and 9–15) revealed KLK5 inhibition by all fragments included in the study, excluding D1. D8–11 exhibited the strongest inhibition towards trypsin-like KLK5 and 14, with low K_i values of 3.7 and 3.1 nM, respectively, and much lower inhibition of the chymotrypsin-like KLK7, with K_i of 34.8 nM (Deraison et al., 2007). These results are consistent with the findings of Borgono et al. (2007) of inhibition of multiple KLKs by D1–8, selective inhibition of KLK5 only by D12–15, and highest inhibition specificity towards KLK5 by D9–12. The strongest inhibitory capacity of multiple LEKTI fragments towards KLK5 supports the paradigm of KLK5 being the initiator of the epidermal KLK activation cascade.

Epidermal pH gradient

The epidermal pH gradient is a critical factor regulating skin barrier homeostasis. SC acidity has been shown to control lipid interactions, corneodesmosomal degradation, and microbial defense by regulating the activity of

lipid-processing enzymes and kallikrein serine proteases. KLK5 and 7 have optimal activity at neutral pH, and retain significant activity at acidic pH, resulting in degradation of CDSN, DSC1, and DSG1 corneodesmosomes (Caubet et al., 2004). Experimental increases in pH lead to higher KLK activity, resulting in over-degradation of SC structural proteins, such as DSG1 corneodesmosome, and of SC lipid-processing enzymes, such as β -glucocerebrosidase, leading to destruction of SC cohesion and lipid permeability barrier, respectively (Hachem et al., 2005).

Epidermal pH regulation of kallikreins is bidirectional, as it modulates both KLK inhibition and activity by regulating the kinetics of the interaction between KLKs and LEKTI inhibitory fragments. A recent *in vitro* study by Deraison et al. (2007) demonstrated that LEKTI fragments D8–11 tightly bind to KLK5 and form stable complexes at pH 7.5, which mimics the pH of the SC/SG border. Increased dissociation of KLK5 from LEKTI fragments occurs with a decrease in pH from 7.5 to 4.5, akin to the SC pH gradient. Thus, the processes of KLK binding to LEKTI fragments in the deeper SC and release of free KLKs in the superficial SC are intrinsically governed by the decreasing pH gradient along the SC (Figure 2). The removal of KLK inhibition, combined with the retention of KLK proteolytic activity at acidic SC pH of 5.5–4.5, leads to regulated corneodesmosomal degradation and proper skin desquamation from the superficial SC layer (Caubet et al., 2004; Deraison et al., 2007). The pH-dependent regulation of KLK activity implies that KLKs are involved in SC homeostatic barrier functions other than desquamation, such as antimicrobial processing and lipid barrier formation.

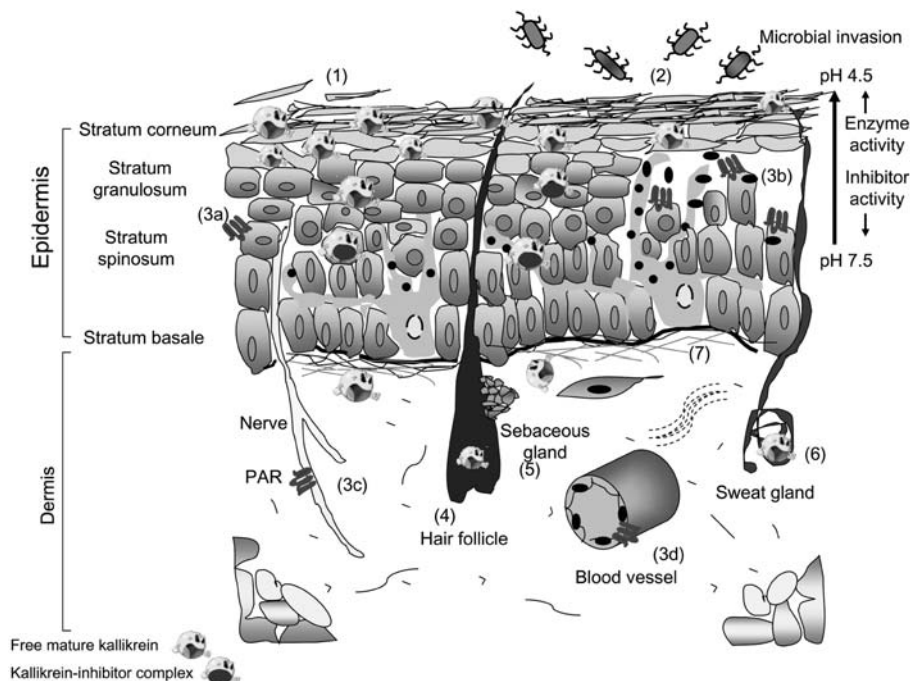


Figure 2 Schematic representation of putative PAR- and non-PAR-mediated kallikrein functions in the skin.

(1) Skin desquamation; (2) antimicrobial activation upon microbial invasion; (3a) PAR-mediated inflammation; (3b) PAR-mediated pigmentation; (3c) PAR-mediated pain induction; (3d) PAR-mediated vasodilation; (4) hair maturation; (5) sebum production; (6) sweat production; (7) ECM degradation. The epidermal pH gradient governing regulated release of active KLKs from the KLK-inhibitor complex into the SC is shown on the right.

Roles of kallikreins in normal skin physiology

The premise of kallikrein actions in the human epidermis is that these proteases may be promiscuously capable of direct or indirect targeting of different epidermal substrates and/or receptors, thus regulating important physiological processes in the skin.

Skin desquamation

Desquamation is an active process whereby humans shed between 2×10^8 and 10×10^8 cells per day (Milstone, 2004). This homeostatic process balances the *de novo* production of squamous keratinocytes in the basal layer with the elimination of corneocytes at the skin surface. Regulated corneocyte shedding upon corneodesmosomal degradation underlies proper desquamation. Corneodesmosomes are structural proteins with similar morphology to the desmosomes maintaining adhesion between neighboring keratinocytes in lower epidermal layers. However, corneodesmosomes contain a characteristic adhesive glycoprotein at their surface, known as corneodesmosin (CDSN), and attach neighboring corneocytes in the upper SC layer. An epidermal expression gradient of seven adhesion proteins (DSG1–4 and DSC1–3) has been reported, whereby DSG1, DSG4, and DSC1 are highly expressed in the SC and are referred to as corneodesmosomes. Conversely, DSG3, DSC2, and DSC3 desmosomes are highly expressed in the SB layer, whereas DSG2 is solely expressed there (Green and Simpson, 2007).

Skin desquamation is pH-dependent and is suggested to occur via initial proteolysis of non-peripheral corneodesmosomes at the transition from the inner stratum compactum to the outer stratum disjunctum of the SC, resulting in retention of corneodesmosomes at the lateral edges of corneocytes. These corneodesmosomes are degraded in the superficial SC, leading to corneocyte shedding (Ishida-Yamamoto et al., 2005). Epidermal kallikreins such as KLK5 and KLK7 degrade DSG1, and DSC1 corneodesmosomal isoforms (Descargues et al., 2006; Borgono et al., 2007), as well as their CDSN glycoprotein (Simon et al., 2001). Furthermore, Borgono et al. (2007) have recently characterized KLK6, 13, and 14 as potential desquamatory enzymes, since they digest DSG1 and/or are inhibited by the epidermal KLK inhibitor LEKTI.

Skin permeability barrier

Skin barrier function depends on the formation of mature lamellar membranes in the SC subsequent to proper extracellular lipid processing of lipid precursors secreted by LGs. As indicated in Table 1, lipid precursors such as glycosylceramides, sphingomyelin, and phospholipids are released into the SG/SC interface for processing by β -glucocerebrosidase, acidic sphingomyelinase, and secretory phospholipase A2 into ceramides and free fatty acids. The resulting lipids, particularly ceramides, are essential, as they form extended hydrophobic lamellar sheets in SC extracellular spaces, limiting water and electrolyte loss and thereby composing the skin's lipid permeability barrier (Hafttek, 2002; Hachem et al., 2006).

Lamellar membrane organization is a pH-dependant process, as studies have shown that lipid processing enzymes exhibit optimal activity at acidic pH (Hachem et al., 2003). Experimental increases in pH induce lipid-processing defects visualized as the formation of immature lamellar membranes in the SC. It has been suggested that such pH-induced lipid barrier dysfunction is mediated by the actions of human tissue kallikreins (Hachem et al., 2003, 2006), as KLKs may regulate lamellar membrane formation via proteolytic degradation of SC lipid-processing enzymes (Hachem et al., 2003) or down-regulation of their secretion by LGs (Hachem et al., 2006). Earlier studies showed that incubation of skin extracts with active recombinant KLK7 at an elevated pH of 7.6 resulted in decreased immunoblotting of both β -glucocerebrosidase and acidic sphingomyelinase lipid-processing enzymes (Hachem et al., 2003). However, later studies by the same group revealed that inhibition of serine protease activity led to permeability barrier recovery by enhancing LG secretion of lipids (Hachem et al., 2006). Hence, the current premise suggested by Hachem et al. is that LG secretion, not lipid processing, is down-regulated by pH-induced increases in kallikrein activity, leading to barrier disruption. They suggest that deregulation of LG lipid secretion occurs via kallikrein-mediated activation of PARs (Hachem et al., 2006).

Proteolytic processing of antimicrobial peptides

Epidermal keratinocytes synthesize and secrete antimicrobial peptides that harbor the skin's innate immunity against bacterial, fungal, and viral infections. β -Defensins and cathelicidins are two major antimicrobial peptide families expressed by keratinocytes and neutrophils; β -defensins are constitutively expressed (Ong et al., 2002), and cathelicidins are induced and deposited at inflammation sites upon infection (Ong et al., 2002; Braff et al., 2005). Tight control of cathelicidin peptide expression is required to ensure their activity when defense against microbial invasion is required. Cathelicidins comprise a conserved N-terminal cathelin pro-domain and a variable C-terminal antimicrobial domain of 30–40 amino acids that becomes active after cleavage (Niyonsaba and Ogaawa, 2005). KLK5 and 7 regulate cathelicidin pro-inflammatory activity by processing either the nascent pro-cathelicidin (hCAP18) or the mature peptide form (LL-37), serving as activators and inactivators. hCAP18 is biologically inactive (Zaiou et al., 2003). KLK processing of hCAP18 to active antimicrobial peptide forms, such as LL-37, stimulates host-cell inflammatory reactions in response to infection, as LL-37 is known to act as a chemoattractant of neutrophils, monocytes, mast cells and T-cells upon tissue insult (Yamasaki et al., 2006). Subsequent to resolving the microbial challenge, KLK5 and 7 process LL-37 to peptide forms that lack pro-inflammatory activity (Yamasaki et al., 2006), bringing the SC back to its normal immuno-barrier setting. Furthermore, it has been shown that cathelicidin processing is altered *in vivo* in the absence of the epidermal serine protease inhibitor LEKTI (Yamasaki et al., 2006), suggesting LEKTI involvement in antimicrobial peptide processing via its inhibitory effect on kallikreins.

ECM degradation

The physiological roles of kallikreins extend beyond the upper SC and the SG layers to encompass the whole epidermis. In addition to degrading corneodesmosomes in the upper epidermis, KLKs are capable of degrading adhesion molecules in the basement membrane of the lower epidermis and ECM. ECM and basement membranes can be proteolysed by serine proteases, cysteine proteases, aspartic proteases, and MMPs. KLK5, 6, 13, and 14 have been shown to cleave laminin, fibronectin, and collagen I–IV *in vitro* (Borgono and Diamandis, 2004; Ghosh et al., 2004; Kapadia et al., 2004).

Complete loss of cell-stromal adhesion allows malignant cells to detach from primary tumor sites to enter the circulation, extravasate, and invade other tissues, leading to tumor metastasis. ELISA of culture supernatants from breast cancer epithelial cells revealed that these cells secrete abundant KLK6 in addition to MMPs (Ghosh et al., 2004). KLK6 overexpression is linked to cancer progression as it enhances degradation the basal membrane and ECM. Elevated *in vivo* KLK6 expression was recently detected in malignant squamous cell carcinoma leading to enhanced proteolysis of the extracellular domain of E-cadherin, a process known as ectodomain shedding (Klucky et al., 2007). KLK6 overexpression was proven to inhibit E-cadherin function resulting in accelerated tumor cell metastasis (Klucky et al., 2007).

Studies have previously shown that MMP inhibitors block MMP-induced desmosome degradation, strengthening cell-cell adhesion in squamous carcinoma cells (Johansson et al., 2000). Interestingly, the actions of overexpressed KLK6 can be inhibited by tissue inhibitors of metalloproteinase TIMP-1 and TIMP-3, rescuing squamous carcinoma cell adhesion defects *in vitro* (Klucky et al., 2007), suggesting the existence of cross-talk between KLK6, MMPs, and their tissue inhibitors TIMPs. Tissue kallikreins, particularly KLK6, are considered potential candidates for epithelial anti-metastatic therapy.

PAR-mediated effects

PARs 1–4 are members of the seven-transmembrane G protein-coupled receptor family, activated by proteases such as trypsin, mast cell tryptase, cathepsin G, and thrombin. PARs are cell surface receptors expressed on keratinocytes (PAR₂), melanocytes (PAR₁), fibroblasts (PAR₂), neurons (PAR₂), and dermal capillaries (PAR₁) (Rattenholl and Steinhoff, 2003). PAR activation occurs intramolecularly by irreversible proteolytic cleavage of the extracellular N-terminal peptide, exposing a tethered ligand that binds the second extracellular loop of the receptor and thus initiates signaling. PARs mediate multiple signaling pathways by coupling to G proteins and stimulating a variety of downstream targets (Dery et al., 1998). Increasing evidence now attests to the role of kallikreins as modulators of PAR signaling. Recent *in vitro* and *in vivo* work by Oikonomopoulou et al. (2006) has demonstrated that PAR activity may be targeted by active KLK5, 6, and 14. KLK5 and KLK6 were shown to activate PAR₂, whereas KLK14 was reported to inactivate PAR₁ and activate PAR₂ and PAR₄.

Among the four PARs, PAR₂ is of prime interest, as it is activated by trypsin cleavage and is co-localized with tissue kallikreins in the SG and in keratinocytes of hair follicles and sebaceous glands. PAR₂ is also present on dermal dendritic cells, with a potential role in pain induction. PAR₂ receptors are attractive research targets for dermatologists and cosmeticians owing to their implication in skin inflammation, cell proliferation, tumor suppression, skin pigmentation, and skin moisture. As activators of PAR₂ receptors, kallikreins are of increasing interest to researchers investigating the above-mentioned skin processes.

In the cosmetics arena, natural non-denatured soybean-derived trypsin inhibitors have been emphasized as major ingredients of cosmetic products targeting skin pigmentation, UV exposure, and skin moisture. The desired effects of these products are attributed to trypsin inhibition leading to blockade of PAR₂ activation. Soybean-derived soy seeds and soymilk contain soybean trypsin inhibitor (STI) and Bowman-Birk inhibitor (BBI), respectively (Paine et al., 2001). It has been reported that negative regulation of PAR₂ activity on keratinocytes by STI and BBI results in diminished keratinocyte-melanocyte interaction, lowering pigment deposition in the upper epidermis and resulting in skin lightening (Paine et al., 2001). It has been suggested that STI reduces UV light-induced skin cancer, as topical application of STI halts tumor progression in mice exposed to UVB for long periods (Huang et al., 2004). It is plausible that cosmetic products containing natural soybean extracts block PAR₂ signaling pathways via kallikrein inhibition, since STI has been proven to inhibit trypsin-like KLK5 and 14 with high efficiency (Brattsand et al., 2005). KLK-PAR-mediated regulation of skin moisture and protection against UV exposure requires further elucidation, as reduced KLK5 and 7 expression in the upper SC of dry skin and elevated KLK activity following UV radiation have been reported (Voegeli et al., 2007).

The roles of kallikreins in the skin seem to be more diverse than previously thought, encompassing signaling networks mediated by specific keratinocyte-surface receptors, such as PAR₂, in addition to proteolytic activation cascades and cross-talk with epidermal kallikreins and/or other epidermal proteases. Emerging data suggest KLK presence in the dermis: Komatsu et al. (2003, 2005b) have identified several KLK mRNAs and proteins in sebaceous glands, eccrine sweat glands, hair follicles and nerves, including KLK5, 6, 7, and 14. The ability of some of these KLKs to cleave ECM proteins also indicates their potential presence in lower epidermal and upper dermal layers. Although much is known about KLK roles in the upper epidermis, much more research in terms of the characterization and experimental validation of KLK localization and potential functions in the human dermis is required. A list of the putative PAR and non-PAR mediated roles of human tissue kallikreins in the skin is shown in Figure 2.

Kallikreins in skin diseases

Aberrations in kallikrein levels and/or activity have been detected in inflammatory skin diseases such as atopic

Table 3 Summary of common KLK, LEKTI, and PAR2 epidermal aberrations associated with skin diseases.

Skin disease	KLK			LEKTI			PAR ₂		
	Disease pathogenesis	Levels	Activity	Localization	Levels	Activity	Localization	Levels	Localization
Atopic dermatitis (OMIM 603165)	Chronic inflammatory, dry, itchy, allergic skin disease involving immune, endocrine, metabolic and infectious factors	Higher trypsin- and chymotrypsin-like KLKs (Komatsu et al., 2007a)	No significant difference from normal (Komatsu et al., 2007a)	Expanded in upper epidermis only (Komatsu et al., 2007a)	-	-	SG	Higher (Descargues et al., 2006)	Expanded lower and co-localized with KLKs (Komatsu et al., 2007a)
Psoriasis vulgaris (OMIM 177900)	Chronic inflammatory dermatosis characterized by erythematous plaques and hyperproliferative keratinocyte activity	Higher trypsin- and chymotrypsin-like KLKs (Komatsu et al., 2007b)	Hyperactive KLK7 (Komatsu et al., 2007b)	Expanded lower in the epidermis (Komatsu et al., 2007b)	-	-	SG	Higher	-
Netherton syndrome (OMIM 256500)	Autosomal recessive mutation in <i>SPINK5</i> gene on chromosome 5q32 causing truncation and/or loss of the epidermal serine protease inhibitor LEKTI	Higher trypsin- and chymotrypsin-like KLKs (Komatsu et al., 2002)	Hyperactive KLKs due to lack of regulation by LEKTI (Komatsu et al., 2002)	Expanded lower to periphery of keratinocytes in upper spinous layer and glanular layer (Komatsu et al., 2002)	Lower or absent due to absence of <i>SPINK5</i> expression (Bitoun et al., 2003)	Inactive	-	Higher (Descargues et al., 2006)	Expanded lower in an increased number of cell layers from the upper spinous to the granular layer
Peeling skin syndrome (OMIM 609796)	Autosomal recessive genodermatosis with shedding of the outer epidermis	Higher trypsin- and chymotrypsin-like KLKs	Normal or hyperactive (Komatsu et al., 2006a)	Expanded lower in the epidermis (Komatsu et al., 2006a)	Normal or elevated	Normal or hyperactive	Expanded lower in the epidermis	-	-

dermatitis (AD), Netherton syndrome (NS), psoriasis vulgaris, and peeling skin syndrome (PSS), as indicated in Table 3. Accumulation of scales, with increased numbers of persisting corneodesmosomes in the upper SC, has been detected in many xeroses and hyperkeratosis skin states (Haftek, 2002). Clinical studies have shown that abnormal desquamation is a common symptom of several skin diseases caused by skin barrier damage resulting from microbial infection, lipid barrier disruption, mechanical injury, and genetic mutations in natural protease inhibitors such as LEKTI.

Overexpression of trypsin- and chymotrypsin-like KLK levels (KLK5, 6, 7, 8, 10, 13, and 14) is detected spanning the SC, SG and lower epidermis of AD skin lesions, with chymotrypsin-like elevations being more prominent (Komatsu et al., 2007a). However, KLK overexpression in AD does not translate into any significant increase in SC trypsin- or chymotrypsin-like activity (Komatsu et al., 2007a), presenting a puzzling observation that remains to be explained. Alternatively, KLK7 hyperactivity and elevated levels of many trypsin-like KLKs are detected in psoriasis, in addition to higher levels of certain trypsin-like KLKs (KLK6, 10, and 13) in non-lesional SC of psoriatic patients (Komatsu et al., 2007b). Similarly, KLK overexpression is detected in the SC of PSS patients (Komatsu et al., 2006a).

The paramount importance of maintaining a physiological regulatory balance between KLKs and their epidermal inhibitors is demonstrated in the devastating skin disease NS, in which severe ichthyosiform erythroderma, 'bamboo hair', and atopic diathesis symptoms occur as a result *SPINK5* mutations, leading to loss or truncation of LEKTI (Chavanas et al., 2000; Sprecher et al., 2001; Descargues et al., 2005). These skin barrier dysfunction symptoms are possibly mediated by KLK hyperactivity in the LEKTI-free NS epidermis (Komatsu et al., 2002).

PAR₂ receptors are overexpressed in the epidermis of AD and NS skin lesions and exhibit similar co-localization as human tissue kallikreins (Descargues et al., 2006; Komatsu et al., 2007a), which suggests KLK-PAR co-regulation and involvement in the pathogenesis of these diseases. It has been suggested that KLKs induce inflammation in these skin disorders via PAR₂ activation, in addition to inducing sweat-mediated itch in AD (Stefansson et al., 2006). The common aberrations of KLK, LEKTI, and PAR₂ expression and/or activity in the epidermis of AD, PV, NS, and PSS patients are summarized in Table 3.

Future aspects

Recent findings have reshaped our perception of kallikrein functions in skin physiology, with the realization that this family of enzymes may have cutaneous roles that expand beyond direct proteolytic processing of corneodesmosomes and antimicrobial peptides to indirect targeting of PAR signaling pathways controlling other skin-related processes, such as lipid barrier function, pigmentation, and tumor suppression. The understanding and dissemination of current knowledge about tissue kallikreins in the skin will greatly assist in moving towards

elucidation of KLK signaling networks in the epidermis. Future avenues of kallikrein research in the field of dermatology and skin biology could lead to novel advances in skin disease therapeutics and skin care products.

References

- Bernard, D., Mehul, B., Thomas-Collignon, A., Simonetti, L., Remy, V., Bernard, M.A., and Schmidt, R. (2003). Analysis of proteins with caseinolytic activity in a human stratum corneum extract revealed a yet unidentified cysteine protease and identified the so-called 'stratum corneum thiol protease' as cathepsin L2. *J. Invest Dermatol.* 120, 592–600.
- Bitoun, E., Micheloni, A., Lamant, L., Bonnart, C., Tartaglia-Polcini, A., Cobbold, C., Al Saati, T., Mariotti, F., Mazereeuw-Hautier, J., Boralevi, F., et al. (2003). LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum. Mol. Genet.* 12, 2417–2430.
- Borgono, C.A. and Diamandis, E.P. (2004). The emerging roles of human tissue kallikreins in cancer. *Nat. Rev. Cancer* 4, 876–890.
- Borgono, C.A., Michael, I.P., and Diamandis, E.P. (2004). Human tissue kallikreins: physiologic roles and applications in cancer. *Mol. Cancer Res.* 2, 257–280.
- Borgono, C.A., Michael, I.P., Komatsu, N., Jayakumar, A., Kapadia, R., Clayman, G.L., Sotiropoulou, G., and Diamandis, E.P. (2007). A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. *J. Biol. Chem.* 282, 3640–3652.
- Braff, M.H., Zaiou, M., Fierer, J., Nizet, V., and Gallo, R.L. (2005). Keratinocyte production of cathelicidin provides direct activity against bacterial skin pathogens. *Infect. Immun.* 73, 6771–6781.
- Brattsand, M. and Egelrud, T. (1999). Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. *J. Biol. Chem.* 274, 30033–30040.
- Brattsand, M., Stefansson, K., Lundh, C., Haasum, Y., and Egelrud, T. (2005). A proteolytic cascade of kallikreins in the stratum corneum. *J. Invest. Dermatol.* 124, 198–203.
- Caubet, C., Jonca, N., Brattsand, M., Guerrin, M., Bernard, D., Schmidt, R., Egelrud, T., Simon, M., and Serre, G. (2004). Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *J. Invest. Dermatol.* 122, 1235–1244.
- Chavanas, S., Bodemer, C., Rochat, A., Hamel-Teillac, D., Ali, M., Irvine, A.D., Bonafe, J.L., Wilkinson, J., Taieb, A., Barrandon, Y., et al. (2000). Mutations in *SPINK5*, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25, 141–142.
- Clements, J., Hooper, J., Dong, Y., and Harvey, T. (2001). The expanded human kallikrein (KLK) gene family: genomic organisation, tissue-specific expression and potential functions. *Biol. Chem.* 382, 5–14.
- Deraison, C., Bonnart, C., Lopez, F., Besson, C., Robinson, R., Jayakumar, A., Wagberg, F., Brattsand, M., Hachem, J.P., Leonardsson, G., and Hovnanian, A. (2007). LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Mol. Biol. Cell* 18, 3607–3619.
- Dery, O., Corvera, C.U., Steinhoff, M., and Bunnett, N.W. (1998). Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. *Am. J. Physiol.* 74, C1429–C1452.
- Descargues, P., Deraison, C., Bonnart, C., Kreft, M., Kishibe, M., Ishida-Yamamoto, A., Elias, P., Barrandon, Y., Zambruno, G., Sonnenberg, A., and Hovnanian, A. (2005). *Spink5*-deficient mice mimic Netherton syndrome through degradation of des-

- moglein 1 by epidermal protease hyperactivity. *Nat. Genet.* 37, 56–65.
- Descargues, P., Deraison, C., Prost, C., Fraitag, S., Mazereeuw-Hautier, J., D'Alessio, M., Ishida-Yamamoto, A., Bodemer, C., Zambruno, G., and Hovnanian, A. (2006). Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. *J. Invest. Dermatol.* 126, 1622–1632.
- Diamandis, E.P., Yousef, G.M., Clements, J., Ashworth, L.K., Yoshida, S., Egelrud, T., Nelson, P.S., Shiosaka, S., Little, S., Lijja, H., et al. (2000). New nomenclature for the human tissue kallikrein gene family. *Clin. Chem.* 46, 1855–1858.
- Egelrud, T. and Lundstrom, A. (1991). A chymotrypsin-like proteinase that may be involved in desquamation in plantar stratum corneum. *Arch. Dermatol. Res.* 283, 108–112.
- Egelrud, T., Brattsand, M., Kreutzmann, P., Walden, M., Vitzthum, K., Marx, U.C., Forssmann, W.G., and Magert, H.J. (2005). hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. *Br. J. Dermatol.* 153, 1200–1203.
- Ekholm, I.E., Brattsand, M., and Egelrud, T. (2000). Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? *J. Invest. Dermatol.* 114, 56–63.
- Elias, P.M. (1983). Epidermal lipids, barrier function, and desquamation. *J. Invest. Dermatol.* 80 (Suppl.), 44s–49s.
- Elliott, M.B., Irwin, D.M., and Diamandis, E.P. (2006). *In silico* identification and Bayesian phylogenetic analysis of multiple new mammalian kallikrein gene families. *Genomics* 88, 591–599.
- Emami, N. and Diamandis, E.P. (2007). Human tissue kallikreins: a road under construction. *Clin. Chim. Acta* 381, 78–84.
- Franzke, C.W., Baici, A., Bartels, J., Christophers, E., and Wiedow, O. (1996). Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. *J. Biol. Chem.* 271, 21886–21890.
- Ghosh, M.C., Grass, L., Soosaipillai, A., Sotiropoulou, G., and Diamandis, E.P. (2004). Human kallikrein 6 degrades extracellular matrix proteins and may enhance the metastatic potential of tumour cells. *Tumour Biol.* 25, 193–199.
- Green, K.J. and Simpson, C.L. (2007). Desmosomes: new perspectives on a classic. *J. Invest. Dermatol.* 127, 2499–2515.
- Hachem, J.P., Crumrine, D., Fluhr, J., Brown, B.E., Feingold, K.R., and Elias, P.M. (2003). pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J. Invest. Dermatol.* 121, 345–353.
- Hachem, J.P., Man, M.Q., Crumrine, D., Uchida, Y., Brown, B.E., Rogiers, V., Roseeuw, D., Feingold, K.R., and Elias, P.M. (2005). Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *J. Invest. Dermatol.* 125, 510–520.
- Hachem, J.P., Houben, E., Crumrine, D., Man, M.Q., Schurer, N., Roelandt, T., Choi, E.H., Uchida, Y., Brown, B.E., Feingold, K.R., and Elias, P.M. (2006). Serine protease signaling of epidermal permeability barrier homeostasis. *J. Invest. Dermatol.* 126, 2074–2086.
- Hadgraft, J. (2002). Crossing the barrier. In: *The Essential Stratum Corneum*, R. Marks, J.L. Leveque and R. Voegeli, eds. (London, UK: Martin Dunitz Ltd.), pp. 103–109.
- Haftek, M. (2002). Ultrastructural aspects of the stratum corneum. In: *The Essential Stratum Corneum*, R. Marks, J.L. Leveque and R. Voegeli, eds. (London, UK: Martin Dunitz Ltd.) pp. 3–16.
- Horikoshi, T., Arany, I., Rajaraman, S., Chen, S.H., Brysk, H., Lei, G., Tyring, S.K., and Brysk, M.M. (1998). Isoforms of cathepsin D and human epidermal differentiation. *Biochimie* 80, 605–612.
- Horikoshi, T., Igarashi, S., Uchiwa, H., Brysk, H., and Brysk, M.M. (1999). Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. *Br. J. Dermatol.* 141, 453–459.
- Huang, M.T., Xie, J.G., Lin, C.B., Kizoulis, M., Seiberg, M., Shapiro, S., and Conney, A.H. (2004). Inhibitory effect of topical applications of nondenatured soymilk on the formation and growth of UVB-induced skin tumors. *Oncol. Res.* 14, 387–397.
- Huntington, J.A., Read, R.J., and Carrell, R.W. (2000). Structure of a serpin-protease complex shows inhibition by deformation. *Nature* 407, 923–926.
- Ishida-Yamamoto, A., Deraison, C., Bonnart, C., Bitoun, E., Robinson, R., O'Brien, T.J., Wakamatsu, K., Ohtsubo, S., Takahashi, H., Hashimoto, Y., et al. (2005). LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. *J. Invest. Dermatol.* 124, 360–366.
- Ishida-Yamamoto, A., Simon, M., Kishibe, M., Miyauchi, Y., Takahashi, H., Yoshida, S., O'Brien, T.J., Serre, G., and Iizuka, H. (2004). Epidermal lamellar granules transport different cargoes as distinct aggregates. *J. Invest. Dermatol.* 122, 1137–1144.
- Johansson, N., Ahonen, M., and Kahari, V.M. (2000). Matrix metalloproteinases in tumor invasion. *Cell. Mol. Life Sci.* 57, 5–15.
- Kapadia, C., Ghosh, M.C., Grass, L., and Diamandis, E.P. (2004). Human kallikrein 13 involvement in extracellular matrix degradation. *Biochem. Biophys. Res. Commun.* 323, 1084–1090.
- Kligman, A.M. (2006). A brief history of how the dead stratum corneum became alive. In: *Skin Barrier*, P.M. Elias and K.R. Feingold, eds. (New York, USA: Taylor & Francis), pp. 15–23.
- Klucky, B., Mueller, R., Vogt, I., Teurich, S., Hartenstein, B., Breuhahn, K., Flechtenmacher, C., Angel, P., and Hess, J. (2007). Kallikrein 6 induces E-cadherin shedding and promotes cell proliferation, migration, and invasion. *Cancer Res.* 67, 8198–8206.
- Komatsu, N., Takata, M., Otsuki, N., Ohka, R., Amano, O., Takehara, K., and Saijoh, K. (2002). Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. *J. Invest. Dermatol.* 118, 436–443.
- Komatsu, N., Takata, M., Otsuki, N., Toyama, T., Ohka, R., Takehara, K., and Saijoh, K. (2003). Expression and localization of tissue kallikrein mRNAs in human epidermis and appendages. *J. Invest. Dermatol.* 121, 542–549.
- Komatsu, N., Saijoh, K., Sidiropoulos, M., Tsai, B., Levesque, M.A., Elliott, M.B., Takehara, K., and Diamandis, E.P. (2005a). Quantification of human tissue kallikreins in the stratum corneum: dependence on age and gender. *J. Invest. Dermatol.* 125, 1182–1189.
- Komatsu, N., Saijoh, K., Toyama, T., Ohka, R., Otsuki, N., Husack, G., Takehara, K., and Diamandis, E.P. (2005b). Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. *Br. J. Dermatol.* 153, 274–281.
- Komatsu, N., Suga, Y., Saijoh, K., Liu, A.C., Khan, S., Mizuno, Y., Ikeda, S., Wu, H.K., Jayakumar, A., Clayman, G.L., et al. (2006a). Elevated human tissue kallikrein levels in the stratum corneum and serum of peeling skin syndrome-type B patients suggests an over-desquamation of corneocytes. *J. Invest. Dermatol.* 126, 2338–2342.
- Komatsu, N., Tsai, B., Sidiropoulos, M., Saijoh, K., Levesque, M.A., Takehara, K., and Diamandis, E.P. (2006b). Quantification of eight tissue kallikreins in the stratum corneum and sweat. *J. Invest. Dermatol.* 126, 925–929.
- Komatsu, N., Saijoh, K., Kuk, C., Liu, A.C., Khan, S., Shirasaki, F., Takehara, K., and Diamandis, E.P. (2007a). Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. *Exp. Dermatol.* 16, 513–519.
- Komatsu, N., Saijoh, K., Kuk, C., Shirasaki, F., Takehara, K., and Diamandis, E.P. (2007b). Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy. *Br. J. Dermatol.* 156, 875–883.

- Kurlender, L., Borgono, C., Michael, I.P., Obiezu, C., Elliott, M.B., Yousef, G.M., and Diamandis, E.P. (2005). A survey of alternative transcripts of human tissue kallikrein genes. *Biochim. Biophys. Acta* 1755, 1–14.
- Lundstrom, A. and Egelrud, T. (1988). Cell shedding from human plantar skin *in vitro*: evidence of its dependence on endogenous proteolysis. *J. Invest. Dermatol.* 91, 340–343.
- Lundstrom, A. and Egelrud, T. (1991). Stratum corneum chymotryptic enzyme: a proteinase which may be generally present in the stratum corneum and with a possible involvement in desquamation. *Acta Dermatol. Venereol.* 71, 471–474.
- Lundwall, A., Band, V., Blaber, M., Clements, J.A., Courty, Y., Diamandis, E.P., Fritz, H., Lilja, H., Malm, J., Maltais, L.J., et al. (2006). A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. *Biol. Chem.* 387, 637–641.
- Milstone, L.M. (2004). Epidermal desquamation. *J. Dermatol. Sci.* 36, 131–140.
- Niyonsaba, F. and Ogawa, H. (2005). Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents β -defensins, cathelicidin LL-37 and lysozyme. *J. Dermatol. Sci.* 40, 157–168.
- Norlen, L., Al Amoudi, A., and Dubochet, J. (2003). A cryotransmission electron microscopy study of skin barrier formation. *J. Invest. Dermatol.* 120, 555–560.
- Oikonomopoulou, K., Hansen, K.K., Saifeddine, M., Tea, I., Blaber, M., Blaber, S.I., Scarisbrick, I., Andrade-Gordon, P., Cottrell, G.S., Bunnett, N.W., et al. (2006). Proteinase-activated receptors, targets for kallikrein signaling. *J. Biol. Chem.* 281, 32095–32112.
- Ong, P.Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., Gallo, R.L., and Leung, D.Y. (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.* 347, 1151–1160.
- Paine, C., Sharlow, E., Liebel, F., Eisinger, M., Shapiro, S., and Seiberg, M. (2001). An alternative approach to depigmentation by soybean extracts via inhibition of the PAR-2 pathway. *J. Invest. Dermatol.* 116, 587–595.
- Paliouras, M. and Diamandis, E.P. (2006). The kallikrein world: an update on the human tissue kallikreins. *Biol. Chem.* 387, 643–652.
- Petraki, C.D., Papanastasiou, P.A., Karavana, V.N., and Diamandis, E.P. (2006). Cellular distribution of human tissue kallikreins: immunohistochemical localization. *Biol. Chem.* 387, 653–663.
- Raghunath, M., Tontsidou, L., Oji, V., Aufenvenne, K., Schurmeyer-Horst, F., Jayakumar, A., Stander, H., Smolle, J., Clayman, G.L., and Traupe, H. (2004). *SPINK5* and Netherton syndrome: novel mutations, demonstration of missing LEKTI, and differential expression of transglutaminases. *J. Invest. Dermatol.* 123, 474–483.
- Rattenholl, A. and Steinhoff, M. (2003). Role of proteinase-activated receptors in cutaneous biology and disease. *Drug Dev. Res.* 59, 408–416.
- Schechter, N.M., Choi, E.J., Wang, Z.M., Hanakawa, Y., Stanley, J.R., Kang, Y., Clayman, G.L., and Jayakumar, A. (2005). Inhibition of human kallikreins 5 and 7 by the serine protease inhibitor lympho-epithelial Kazal-type inhibitor (LEKTI). *Biol. Chem.* 386, 1173–1184.
- Shaw, J.L. and Diamandis, E.P. (2007). Distribution of 15 human kallikreins in tissues and biological fluids. *Clin. Chem.* 53, 1423–1432.
- Shin, J.S. and Yu, M.H. (2002). Kinetic dissection of α 1-antitrypsin inhibition mechanism. *J. Biol. Chem.* 277, 11629–11635.
- Simon, M., Jonca, N., Guerrin, M., Haftek, M., Bernard, D., Caubet, C., Egelrud, T., Schmidt, R., and Serre, G. (2001). Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation. *J. Biol. Chem.* 276, 20292–20299.
- Sprecher, E., Chavanas, S., DiGiovanna, J.J., Amin, S., Nielsen, K., Prendiville, J.S., Silverman, R., Esterly, N.B., Spraker, M.K., Guelig, E., et al. (2001). The spectrum of pathogenic mutations in *SPINK5* in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J. Invest. Dermatol.* 117, 179–187.
- Stefansson, K., Brattsand, M., Ny, A., Glas, B., and Egelrud, T. (2006). Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. *Biol. Chem.* 387, 761–768.
- Voegeli, R., Rawlings, A.V., Doppler, S., Heiland, J., and Schreier, T. (2007). Profiling of serine proteases activities in the human stratum corneum and detection of a stratum corneum trypsin-like enzyme. *Int. J. Cosmet. Sci.* 29, 191–200.
- Yamasaki, K., Schaubert, J., Coda, A., Lin, H., Dorschner, R.A., Schechter, N.M., Bonnart, C., Descargues, P., Hovnanian, A., and Gallo, R.L. (2006). Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J.* 20, 2068–2080.
- Yoon, H., Laxmikanthan, G., Lee, J., Blaber, S.I., Rodriguez, A., Kogot, J.M., Scarisbrick, I.A., and Blaber, M. (2007). Activation profiles and regulatory cascades of the human kallikrein-related peptidases. *J. Biol. Chem.* 282, 31852–31864.
- Yousef, G.M. and Diamandis, E.P. (2001). The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr. Rev.* 22, 184–204.
- Yousef, G.M., Chang, A., Scorilas, A., and Diamandis, E.P. (2000). Genomic organization of the human kallikrein gene family on chromosome 19q13.3–q13.4. *Biochem. Biophys. Res. Commun.* 276, 125–133.
- Zaiou, M., Nizet, V., and Gallo, R.L. (2003). Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J. Invest. Dermatol.* 120, 810–816.